CLAIM AMENDMENTS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Claims 1-4 (cancelled)

5. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprises:

a) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP); and

ii) a second portion comprising a first target-specific sequence comprising a first base at an interrogation position; and

b) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

wherein if said first base of each said first ligation probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and

wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an adapter sequence, said fifth portion being distinct from said first, second, third or fourth portions of said ligation probes;

c) immobilizing each said ligation complex to a solid support;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

Claims 6-8 (cancelled)

9. (currently amended) The method according to claim 5, 26, 32 and 33 wherein said removing comprises:

a) enzymatically adding a binding ligand to said plurality of target sequences to form a target sequence comprising said binding ligand;

b) binding a hybridization complex comprising said plurality of target sequences comprising said binding ligand to a binding partner immobilized on a solid support;

c) washing away unhybridized probes; and

d) eluting said probe from said solid support.

10. (previously presented) The method according to claim 5, 26, 32, or 33 wherein said removing is done using a double-stranded specific moiety.

11. (previously presented) The method according to claim 10 wherein said double-stranded specific moiety is an intercalator attached to a support.

12. (currently amended) The method according to claim 11 wherein said support is a plurality of beads.

13. (previously presented) The method according to claim 5, 26, 32, or 33 wherein said amplifying is done by:

a) hybridizing a first universal primer to said UUP;

b) providing a polymerase and dNTPs such that said first universal primer is extended;

c) hybridizing a second universal primer to said DUP;

d) providing a polymerase and dNTPs such that said second universal primer is extended; and

e) repeating steps a) through d).

14. (previously presented) The method according to claim 5, 26, 32, or 33 wherein said array comprises:

a) a substrate with a patterned surface comprising discrete sites; and

b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

15. (previously presented) The method according to claim 14 wherein said discrete sites comprise wells.

16. (previously presented) The method according to claim 14 wherein said substrate comprises a fiber optic bundle.

Claims 17-18 (cancelled)

19. (currently amended) The method according to claim 5 or 32, further comprising providing a support on which the plurality of target sequences are ~~is~~ immobilized.

20. (previously presented) The method according to claim 19, wherein said non-hybridized probes are removed without removing said target sequence from said support.

21. (currently amended) The method according to claim 5 or 32, further comprising attaching said plurality of target sequences to a support.

22. (currently amended) The method according to claim 21, wherein said plurality of target sequences are ~~is~~ attached to said support by a method selected from the group consisting of labeling said plurality of target sequences with a functional attachment moiety capable of binding to said support and interacting said functional attachment moiety with said support, absorption of said plurality of target sequences on said support wherein said support comprises charged groups, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.

23. (previously presented) The method according to claim 9, wherein said support is selected from the group consisting of paper, plastic and tubes.

Claims 24-25 (cancelled)

26. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprise:

a) providing a support on which the plurality of target sequences is immobilized;

b) hybridizing a plurality of 96 first ligation probes to said first target domains, each said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP); and

ii) a second portion comprising a first target-specific sequence comprising a first base at an interrogation position; and

c) hybridizing a plurality of 96 second ligation probes to said second target domains, each said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

wherein if said first base of each said first ligation probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an adapter sequence, said fifth portion being distinct from said first, second, third or fourth portions of said ligation probes;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

Claims 27-29 (cancelled)

30. (currently amended) The method according to claim 9 wherein said solid support is a plurality of beads.

31. (currently amended) The method according to claim 26 wherein said non-hybridized probes are removed without removing said plurality of target sequences from said support.

32. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP);

ii) a second portion comprising a first target-specific sequence; and

iii) an interrogation position that is complementary to said detection position;

and

b) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP);

and

ii) a fourth position comprising a second target-specific sequence;

whereby if said interrogation position of each said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an adapter sequence, said fifth portion being distinct from said first, second, third or fourth portions of said ligation probes;

c) immobilizing each said ligation complex to a solid support;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

33. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the plurality of target sequences is immobilized;

b) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probe comprising:

i) a first portion comprising an upstream universal priming site (UUP);

ii) a second portion comprising a first target-specific sequence; and

iii) an interrogation position; and

c) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

whereby if said interrogation position of each said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an adapter sequence, said fifth portion being distinct from said first, second, third or fourth portions of said ligation probes;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

34. (previously presented) The method according to claim 15, wherein said substrate comprises a fiber optic bundle.

35. (currently amended) The method according to claim 22, wherein said plurality of target sequences are ~~is~~ attached to said support by labeling said target sequence with a functional attachment moiety capable of binding to said support and interacting said functional attachment moiety with said support.

36. (currently amended) The method according to claim 22, wherein said plurality of target sequences are ~~is~~ attached to said support by absorption of said target sequence on said support wherein said support comprises charged groups.

37. (currently amended) The method according to claim 22, wherein said plurality of target sequences are ~~is~~ attached to said support by direct chemical attachment of said target sequence to said support.

38. (currently amended) The method according to claim 22, wherein said plurality of target sequences are ~~is~~ attached to said support by photocrosslinking said target sequence to said support.

39. (currently amended) A method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality

comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprises:

a) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP); and

ii) a second portion comprising a first target-specific sequence comprising a first base at an interrogation position; and

b) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

wherein if said first base of each said first ligation probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an exogenous adapter sequence;

c) immobilizing each said ligation complex to a solid support;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

40. (previously presented) The method of claim 39, wherein said exogenous adapter sequence is nested between said first or second portions of said first ligation probe or said third or fourth portions of said second ligation probe.

41. (currently amended) The method according to claim 39, 54, 57 and 58, wherein said removing comprises:

a) enzymatically adding a binding ligand to said plurality of target sequences to form a target sequence comprising said binding ligand;

b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;

c) washing away unhybridized probes; and

d) eluting said probe from said solid support.

42. (previously presented) The method according to claim 39, 54, 57 and 58, wherein said removing is done using a double-stranded specific moiety.

43. (previously presented) The method according to claim 42, wherein said double-stranded specific moiety is an intercalator attached to a support.

44. (currently amended) The method according to claim 43 wherein said support is a plurality of beads.

45. (previously presented) The method according to claim 39, 54, 57 and 58, wherein said amplifying is done by:

a) hybridizing a first universal primer to said UUP;

b) providing a polymerase and dNTPs such that said first universal primer is extended;

c) hybridizing a second universal primer to said DUP;

d) providing a polymerase and dNTPs such that said second universal primer is extended; and

e) repeating steps a) through d).

46. (previously presented) The method according to claim 39, 54, 57 and 58, wherein said array comprises:

a) a substrate with a patterned surface comprising discrete sites; and

b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

47. (previously presented) The method according to claim 46, wherein said discrete sites comprise wells.

48. (previously presented) The method according to claim 46, wherein said substrate comprises a fiber optic bundle.

49. (currently amended) The method according to claim 39 or 57, further comprising providing a support on which the plurality of target sequences are ~~is~~ immobilized.

50. (currently amended) The method according to claim 49, wherein said non-hybridized probes are removed without removing said plurality of target sequences from said support.

51. (currently amended) The method according to claim 39 or 57, further comprising attaching said plurality of target sequences to a support.

52. (currently amended) The method according to claim 51, wherein said plurality of target sequences are ~~is~~ attached to said support by a method selected from the group consisting of labeling said plurality of target sequences with a functional attachment moiety capable of binding to said support and interacting said functional attachment moiety with said support, absorption of said plurality of target sequence on said support wherein said support comprises charged groups, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.

53. (previously presented) The method according to claim 41, wherein said support is selected from the group consisting of paper, plastic and tubes.

54. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within said plurality comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprise:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP); and

ii) a second portion comprising a first target-specific sequence comprising a first base at an interrogation position; and

c) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probe comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

wherein if said first base of each said first ligation probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an exogenous adapter sequence;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

55. (currently amended) The method according to claim 41 wherein said solid support is a plurality of beads.

56. (previously presented) The method according to claim 54 wherein said non-hybridized probes are removed without removing said target sequence from said support.

57. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within

said plurality comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP);

ii) a second portion comprising a first target-specific sequence; and

iii) an interrogation position that is complementary to said detection position;

and

b) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP);

and

ii) a fourth position comprising a second target-specific sequence;

whereby if said interrogation position of each said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an exogenous adapter sequence;

c) immobilizing each said ligation complex to a solid support;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

58. (currently amended) A multiplex method of determining the identification of a nucleotide at a detection position in a plurality of target sequences, each target sequence within

said plurality comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the plurality of target sequences is immobilized;

b) hybridizing a plurality of 96 first ligation probes to said first target domains, each of said first ligation probes comprising:

i) a first portion comprising an upstream universal priming site (UUP);

ii) a second portion comprising a first target-specific sequence; and

iii) an interrogation position; and

c) hybridizing a plurality of 96 second ligation probes to said second target domains, each of said second ligation probes comprising:

i) a third portion comprising a downstream universal priming site (DUP); and

ii) a fourth portion comprising a second target-specific sequence;

whereby if said interrogation position of each said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and

wherein at least one of said first and second ligation probes of each said ligation complex comprises a fifth portion comprising an exogenous adapter sequence;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes of each said ligation complex to form a ligated probe;

f) amplifying each said ligated probe using said UUP and said DUP to generate a plurality of amplicons, wherein said amplicons contain said fifth portion comprising an adapter sequence;

g) contacting said amplicons with an array of capture probes, wherein a capture probe of said array specifically hybridizes to said adapter sequence and is distinct from said target sequence; and

h) determining the nucleotide at each said detection position of said plurality of target sequences.

59. (previously presented) The method of claim 58, wherein said exogenous adapter sequence is nested between said first and second portions of said first ligation probe or said third and fourth portions of said second ligation probe.

60. (previously presented) The method according to claim 47, wherein said substrate comprises a fiber optic bundle.

61. (currently amended) The method according to claim 52, wherein said plurality of target sequences are ~~is~~ attached to said support by labeling said target sequence with a functional attachment moiety capable of binding to said support and interacting said functional attachment moiety with said support.

62. (currently amended) The method according to claim 52, wherein said plurality of target sequences are ~~is~~ attached to said support by absorption of said target sequence on said support wherein said support comprises charged groups.

63. (currently amended) The method according to claim 52, wherein said plurality of target sequences are ~~is~~ attached to said support by direct chemical attachment of said target sequence to said support.

64. (currently amended) The method according to claim 52, wherein said plurality of target sequences are ~~is~~ attached to said support by photocrosslinking said target sequence to said support.